Viridaphin A₁ Glucoside, a Green Pigment Possessing Cytotoxic and Antibacterial Activity from the Aphid *Megoura crassicauda*

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Extraction and Isolation. The aphids (2.1 g) were crushed with a pestle in n-hexane and methanol several times. The combined methanol solutions were evaporated to give crude extracts. The extracts were subjected to reversed-phase silica gel chromatography (20 g, MeOH/H$_2$O=1:1) to afford the green pigment 1 (0.8 mg). To obtain a large amount of pigment 1, we did not attempt to separate the aphid, *M. crassicauda*, from the greenish aphid, *Acrthosiphon pisum*, which were found in close association on *Vicia sativa*, due to the loss incurred by separating these aphids. A mixture of these aphids (total 1.78 Kg) was crushed with a pestle in n-hexane (total 3 L) and methanol (total 7.5 L) several times. The combined methanol solutions were evaporated to give crude extracts. The extracts were subjected to reversed-phase silica gel chromatography (100 g, MeOH/H$_2$O=1:1) and Sephadex LH-20 (MeOH/EtOAc=1:1) several times to afford the green pigment 1 (97.3 mg).

MTT Assay for Cytotoxic Activity. HL-60 (human promyelocytic leukemia-60) cells were grown in suspension culture and incubated at 37 °C in RPMI-1640 medium supplemented with 10% FBS and glutamine (2 mM). The cytotoxicity of 1 in HL-60 cells was analyzed by colorimetric 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay with some modifications.\textsuperscript{15} HL-60 cells (1×10$^4$) were plated on 96-well plates and incubated at 37 °C in 5% CO$_2$/95% atmosphere for 1 h. Then, serially diluted solutions of test compound 1 (10 µL) were added to final concentrations of 0.63 -100 µM. Then, the cells were incubated for 24 h. After 24 h, 10 µL of MTT (5 mg/mL: stock solution) was added, and cells were incubated for an additional 4 h. Next, cells were treated with 100 µL of 20% sodium dodecyl sulfate in 0.01 N HCl to solubilize the intracellular formazan crystals. The optical density (OD) of each well was measured with a microplate spectrophotometer equipped with a 570 nm filter. The absorbances of the sample-treated cells were compared to those of the vehicle (0.5% DMSO-standard medium)-treated control to calculate the percent change. The results of cytotoxic activity are expressed in terms of the IC$_{50}$.

Antimicrobial Assay. The following microorganisms were used to test for antimicrobial activity: *Bacillus subtilis* NBRC 3134, *Staphylococcus aureus* NBRC 5035, *Mycobacterium smegmatis* NBRC 13082, *Klebsiella pneumoniae* NBRC 3512 and *Pseudomonas aeruginosa* NBRC 12582. Bacterial strains were tested in a microdilution assay, and the minimum inhibitory concentration (MIC) values were determined. The bacterial strains were inoculated on YP agar plates (1% polypeptone, 0.2% yeast extract, 0.1% MgSO$_4$·7 H$_2$O, and 2% agar) and were incubated at 30 °C for 12 h. The stock solution of sample was prepared at 10 mg/mL in DMSO and further diluted to varying concentrations in 96 well plates that contained the incubated microbial strains. The plates were incubated at 37 °C overnight and growth inhibition was compared to that caused by varying concentrations of ampicillin (reference).

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